Adaptors and integrators

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The PTB domain expands both the PH-domain set and peptide–protein recognition motifs; the PDZ domain shows an intriguing resemblance.

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The adaptor protein Shc ('Src homology 2, collagen homology') acts to transmit signals from a large class of activated cell surface receptors to Ras-dependent mitogen-activated protein (MAP) kinase, via its phosphorylation, its interaction with Grb2, and the subsequent Sos/Ras pathway. Shc has been shown to possess a novel phosphotyrosine-binding domain (PTB, also known as phosphotyrosine interaction domain, PID), differing in sequence and specificity from its better known SH2 domain. A recent paper [1] describes the structure of the PTB domain complexed with a ligand phosphopeptide, using multidimensional heteronuclear NMR. This landmark paper also identifies a structural and functional similarity between PTB and pleckstrin homology (PH) domains, and suggests a possible connection between signalling-mediated protein-tyrosine kinases, and G-protein-mediated signal transduction. Subsequent papers have confirmed many of these details for the PTB domain from insulin receptor substrate-1 (IRS-1) [2,3]. A similar fold, although differing significantly in detail and topology, is displayed in the structure of a new peptide recognition domain, the PDZ domain [4].

In both the Shc [1], and IRS-1 PTB domains [2,3] (Fig. 1), the 'PH-fold', comprising two medium sized β sheets facing each other at about 60°, with a C-terminal α helix lying at the edges of the sheets, is clearly evident. Peptides form complexes with both proteins by interactions with the β5 sheet and the phosphoryltyrosyl residue is recognized by arginyl residues by coordination and charge neutralization from the guanidino amino groups. The detailed phosphotyrosyl recognition is quite different from that in SH2s [5], and differs between Shc and IRS-1 PTBs, with none of the involved residues conserved between Shc and IRS-1 PTBs. In the crystallographic study of IRS-1 [3], the ligated and unligated forms could be compared, and show essentially complete superimposition, with the exception of the C-terminal helix. This becomes extended by a full turn in the ligated structure and is apparently stabilized by its interactions with the bound phosphopeptide.

The overall precision and accuracy of the NMR structure of Shc PTB [1] is undoubtedly very high, possibly comparable with a 2.0 Å crystal structure. Despite this, the structure, and other NMR structures of SH2 domains with phosphotyrosyl peptides, are intrinsically limited in the description of the phosphotyrosyl-binding pocket. A structure derived from NMR depends heavily on the local density of observable hydrogen atoms, and this is limited around the phosphotyrosyl by the chemical composition of the aromatic ring and bulky phosphate; chemical exchange with solvent water; overlap of chemical shifts of the hydroxyl hydrogens of seryl, the amino and imino hydrogens of the arginyl guanidino group, and backbone amide hydrogens of those residues close to the phosphotyrosine. Mutational analyses [1,2] of arginyl and seryl residues at the phosphotyrosyl site are entirely consistent with the derived structure. They also further support the view that the chemical nature of the coordination and charge interactions between the phosphate and the protein are similar for PTBs and SH2s, even though the topology of the sites and the position of residues are so different. In these PTBs, the peptide ligand is additionally stabilized by forming an additional strand, extending the β sheet to the β5 strand, which is close to the C-terminal α helix.

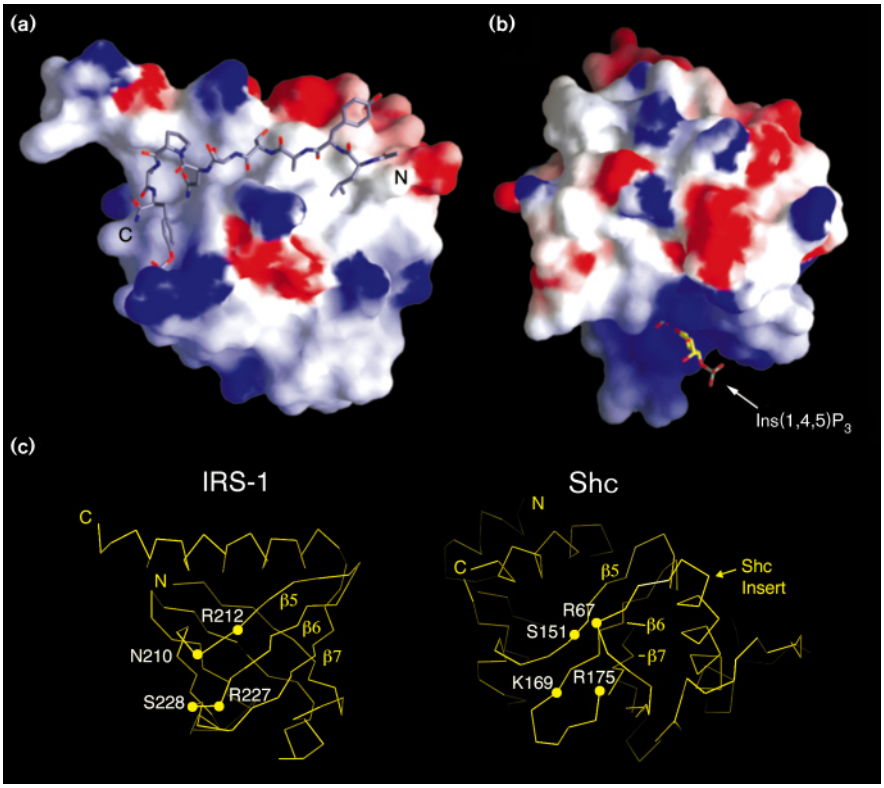
The PDZ domain

PDZs are domains that form components of several proteins involved in synaptic junctions, and have been shown to be responsible for binding to short peptides at the C termini of potassium channels and the *N*-methyl D-aspartate (NMDA) receptor ion channels [4]. The structural basis of peptide recognition, and the protein fold involved, are delineated in a recent paper [4]. Although the connectivity of the elements of secondary structure differ in the PDZ structure, the overall 'PH-like' architecture is retained, and the peptide ligand is bound in a similar position, with a similar, but not identical, β-sheet character (Fig. 2) [4]. As the sequence comparison of PH, PTB, and PDZ domains reveals essentially no correlation [3], the origin and convergence of these architectural features remains a fascinating challenge.

Multiple signals and integrative domains

A casual reader of the signal-transduction literature may be forgiven the impression that everything interacts with everything else. It is therefore natural to approach with caution the interpretation of these observations in terms of functional significance. For the comparison of Shc PTB and PH domains, analysis of the comparable architectural

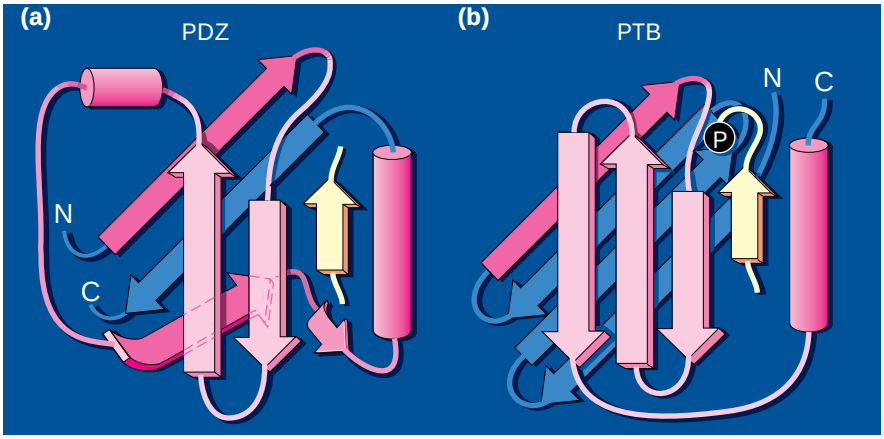
Figure 1



Comparison of the surfaces of a PTB and a PH domain, and backbone traces of the IRS-1 and Shc PTB domain. (a) Molecular surface representation of the IRS-1 PTB domain [3], displayed using GRASP [17]. (b) Surface of the phospholipase Cδ PH domain [8]. (c) The non-conserved recognition sites for phosphotyrosyl on the surface of IRS-1 PTB and Shc PTB [1]. Residues involved in the recognition are identified by the yellow balls, and arise at different sequential positions. (The figure was reproduced from [3], with permission.)

features was extended to show that the micromolar affinities of acidic phospholipids [1] were similar for several PTB and PH domains. This simultaneous similarity of structure and biochemical activity is of compelling interest in the quest to understand the possible inter-relationships of the PTB and PH domains. A possible common feature is recruitment to membranes and localization at positions of high local concentration of acidic phospholipids, which might result from phosphorylation of inositide headgroups by phosphatidyl inositol kinases. One problem with this hypothesis had been the lack, to date, of a well-defined binding site for acidic phospholipids on PH domains: the areas identified as interacting with inositide phosphates differ somewhat in their position in various different PH domains (pleckstrin [6], spectrin [7], PLC-δ [8], and dynamin [9]). In spite of this, all the putative sites do involve residues associated with loops in the N-terminal half of the molecules. As pointed out [1], maybe this

Figure 2



Schematic drawings of (a) PDZ domain [4] and (b) IRS-1 PTB domain [2]. In both cases, peptidic ligands make multiple hydrogen bonds to the adjacent β strand. In the case of PDZ, the C-terminal carboxylate of the peptide is also specifically recognized; in the PTB domains, a β turn followed by the phosphotyrosine is recognized. The N and C termini of the two domains are in quite different locations. (The figure was reproduced from [4], with permission.)

interaction, and the putatively similar one of the PTB, with acidic phospholipids only provides a weak localization signal comparable in intensity with those of farnesylation or myristoylation: several such interactions may be needed to form a multimolecular complex at the membrane inner surface. Such a picture is supported by the activation of Ras by Sos molecules that have been modified by myristolyation or farnesylation [10], and this interaction is augmented by deletion of the C terminus of Sos at the Grb2-binding site. The Shc–Grb2–Sos complex may then be envisaged, by analogy, as providing a localization signal that neutralizes the solubilizing, hence delocalization, effect of the C terminus of Sos.

To add to the complexity, it is also observed [1] that the presence of the phosphopeptide ligand decreases the affinity of PTB for acidic phospholipids. This would be expected if the acidic phospholipids bound directly to the same site as the phosphopeptide, but this seems unlikely given the distance between the areas possibly involved in the two activities (Fig. 3a,b; Fig. 1a,b.). If the role of the Shc PTB domain is to provide a processive phosphorylation [11] by recruitment to a kinase, and subsequent dissociation of the product, then the binding site for phosphopeptides might possess a dual functionality in that it may bind phospholipids as well. A more direct observation of cross interactions between phospholipids and phosphopeptides had been made for the SH2 domain of phosphatidyl inositol-3′-kinase [12], so this class of cross interactions is likely to receive significant attention.

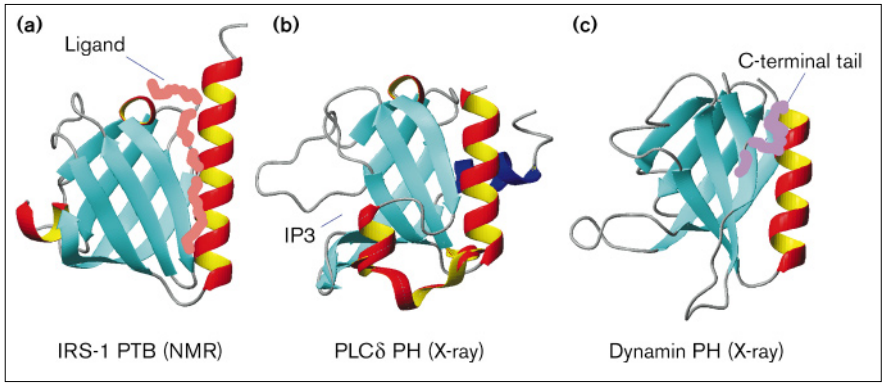
PH domains (or a subclass of them) bind not only to phospholipids but also to $G_{\beta,\gamma}$ proteins ([13], and references therein). Fesik *et al.* [1] extend their hypothesis of the similarity between PTBs and PH domains further in suggesting an integrative point, involving PTBs, between the receptor-tyrosine-kinase pathways, and G-coupled activation of the kinases. For MAP kinase, this hypothesis then provides a physical basis for the observations that Shc is indeed phosphorylated downstream of G_i [14], apparently by $G_{\beta,\gamma}$ association with Shc [14]. Similarly, the association and phosphorylation of Shc associated with chemoattractant receptors, members of the G-protein-coupled heptahelical receptor family, provides a pathway for Lyn activation in neutrophiles [15]. An associated kinase (PYK2) may be required for this interaction [16]. In Figure 3, the structures of IRS-1 PTB (for which the family likeness to PH domains is more readily evident than for Shc PTB), the PH domain of phospholipase C δ (PLCδ) (in which there is a high-affinity site for inositol [1,4,17] trisphosphate), and the dynamin PH domain are compared.

There are some obvious questions and hypotheses of a structural kind. Is the peptide-ligation site a common feature of many of these domains? Can it be blocked, possibly completely, by the C terminus beyond the C-terminal helix (as apparently occurs in dynamin)? Is the acidic-phospholipid-binding site in a defined area, and can its signal integration be either synergistic [18] or antagonistic [1]? Are the highly flexible loops and other insertions to the basic fold functionally significant, or are they vestigial appendices?

These considerations, and other more functional ones, raise the question as to whether the multiple recognition functions widely ascribed to adaptors, incorporating several kinds of recognition domains, can be also ascribed to single domains. These domains may then serve as integrators of several signals, such as acidic phospholipids and peptide ligands. An important test of the maturity of structural biology is its ability not only to provide three-dimensional information about molecular organization, but also to translate that information into functional and integrative hypotheses which can be tested by genetic, physiological, and biochemical experiments. These new studies on PTBs, PDZs, and previous studies of PH domains, surely meet this test.

Figure 3

Comparative topologies of PTB and PH domains. (a) Overall fold of the IRS-1 PTB domain, with its ligand superimposed in pale red. (b) The overall fold of the PLCδ PH domain, with the inositol [1,4,5] triphosphate site, similar to a probable binding site for phospholipids indicated. (c) The X-ray structure of the dynamin PH domain [19], in which the C-terminal extension forms a mini β strand close to the nominal ligand position in Shc PTB. (Produced using the program MOLMOL [20].)



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